REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 2004	Annual (1 Jan 2003 - 31 Dec 2003)

4. TITLE AND SUBTITLE
CHK2, A Candidate Prostate Cancer Susceptibility Gene

5. FUNDING NUMBERS
DAMD17-02-1-0093

6. AUTHOR(S)
Wanguo Liu, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Mayo Clinic, Rochester
Rochester, Minnesota 55905

E-Mail: liu.wanguo@mayo.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

To identify prostate cancer susceptibility genes, we applied a mutation screening of candidate gene approach. In our last year's reporter, we identified a total of 28 (4.8%) germline CHEK2 mutations in 178 clinic tumors, 149 familial prostate cancer (HPC) families, and 400 sporadic cases. Sixteen of the 18 unique CHEK2 mutations identified in this study were not detected among 423 unaffected men, suggesting a pathological effect of CHEK2 mutations in prostate cancer development. In this year's reporter, we identified two somatic CHK2 mutations in 84 clinic prostate tumor samples indicating that CHK2 mutation in prostate cancer could be both germline or somatic. To investigave the function of these mutations in prostate tumorigenesis, we generated stable cell lines and analyzed the CHK2 kinase activities in 9 of the mutants before and after irradiation. While most of the mutations have modest reduced CHK2 kinase activity in comparison with wild-type CHK2, one somatic mutation (Glu321Lys) totally abolishes kinase activity. Our data provide first evidence that the CHK2 mutations identified in prostate cancer, both germline and somatic, impair CHK2 kinase activity suggesting that mutations in CHK2 may contribute to the development of prostate cancer through altering CHK2 kinase activity.

14. SUBJECT TERMS
CHK2, prostate cancer, and susceptibility

15. NUMBER OF PAGES
27

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

AD_____

Award Number: DAMD17-02-1-0093

TITLE: CHK2, A Candidate Prostate Cancer Susceptibility Gene

PRINCIPAL INVESTIGATOR: Wanguo Liu, Ph.D.

CONTRACTING ORGANIZATION: Mayo Clinic, Rochester
Rochester, Minnesota 55905

REPORT DATE: January 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Annual report
DOD grant DAMD17-02-1-0093
***CHK2*, A Candidate Prostate Cancer Susceptibility Gene**
Wanguo Liu, Ph.D.
Mayo Clinic

Introduction:

Prostate cancer is the second most common cancer and the second leading cause of cancer mortality in American men. Previous studies of family history and twins with prostate cancers have shown that genetics plays a critical role in the development of this cancer. However, genetic components contributing to prostate cancer (MIM 300200) have been difficult to identify, largely due to the complexity of this disease and the presence of phenocopies in high-risk families. Regarding to the difficulties to identify high penetrant genes based on linkage analysis and positional cloning, it has been suggested that the pathogenesis of the disease is related at least, in part, to genomic mutations in multiple low-penetrant genes. Although less penetrant, such genes might play an important role at a population level. Our laboratories have recently applied a new approach to identify prostate cancer susceptibility gene(s) based on mutation screening of candidate genes involved in the DNA damage-signaling pathway and identified mutations in the ***CHK2*** gene. CHK2 is a key regulator in this pathway. It regulates a number of downstream effector proteins such as p53 and plays essential roles in coordinating DNA repair, cell cycle progression, transcriptional regulation and apoptosis in response to various DNA-damaging events. Because mutations in p53 is infrequent in prostate cancer, while common (more than 50%) in all other cancers, we **hypothesize** that CHK2, the upstream regulator of p53, could be a candidate prostate cancer susceptibility gene. Therefore, we **propose** to: 1) screen for *CHK2* mutations in 163 familial prostate cancer families, collected at the Mayo Clinic, and determine whether the mutations segregate with prostate cancer in families; 2) perform functional analyses to determine the impact of mutant CHK2 in the DNA damage-signaling pathway using a kinase activity assay; and 3) perform loss of heterozygosity (LOH) studies to determine if CHK2 functions as a tumor suppressor in prostate cancer. These results will advance our understanding of the etiology of prostate cancer and may also enable us to develop diagnostic tools for the early detection and prevention of prostate cancer.

Body:

In year one, we accomplished tasks 1-4 of the proposal. The task 5, which we proposed to fulfil in year two, and the accomplishments associated with this task are summarized below:

Task 5. Kinase activity analyses will be performed on all of the mutations identified including those identified in the familial prostate cancer families. According to the frequency of *CHK2* mutation identified in primary prostate cancer tissues, we may identify more than 20 different mutations in familial cases. So we need to analyze about 20-30 mutations. This work will be done in Months 17-28.

a) Create CHK2 mutants using mutagenesis kit and clone them into PDEST20 vector.
b) Transfect the mutants into an insect cell line (Sf9) to generate recombinant baculovirus encoding GST-fused wild-type and mutants of CHK2.
c) Purify protein using glutathione affinity chromatography.
d) Kinase activity will be measured using GST-Cdc25C as substrate.

As we showed in the report last year, we have identified a total of 18 unique germline CHK2 mutations in the analyses of the sporadic and familial prostate cancer samples (1). In addition, we identified two novel somatic CHK2 mutations in two prostate tumor samples this year (A349G, Arg117Gly and G961A, Glu321Lys). Thus, CHK2 mutations in prostate cancer could be either germline mutations or somatic mutations although the majority of them are germlines.

Since some of the mutaitons have been analyzed and reported previously by us or others, four of them have the same changes in the neighbouring Arg amino acids (Arg180Cys and Arg181Cys), and three truncating mutations obviously abolish kinase activity due to the deletion of the kinase domain (245del15bp, IVS2+1G->A, and Glu239Stop), we focused our effort on the functional analysis of the remaining 12 mutations (Table 1).

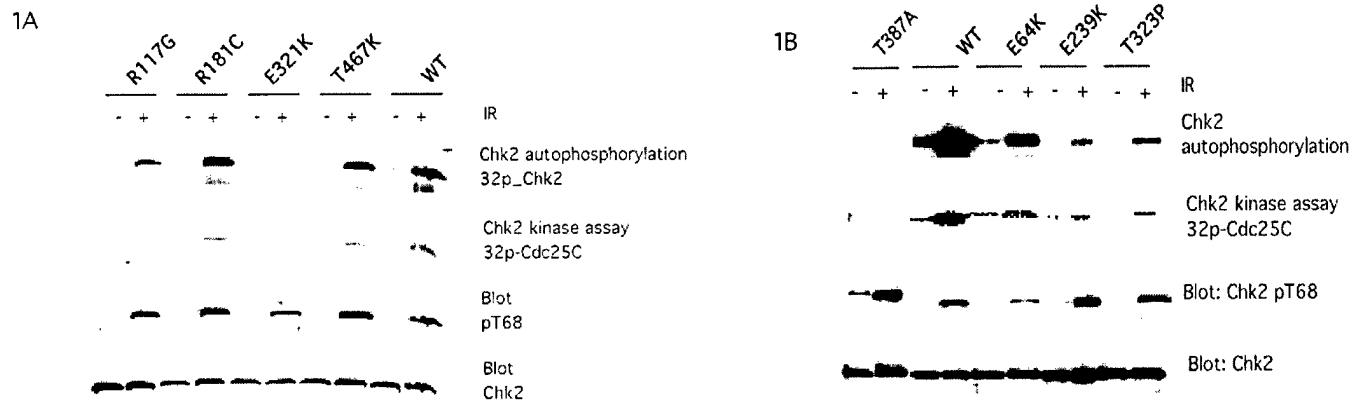
Table 1
CHK2 mutations identified in prostate cancer for kinase activity assay

mutants	Mutation	Amino acid change	Domain	
1	G190A	Glu64Lys	S/TQ-rich	germline
2	A349G	Arg117Gly	FHA	somatic
3	G434C	Arg145Pro	FHA	germline
4	T479C	Ile157Thr	FHA	germline
5	G499A	Gly167Arg	FHA	germline
6	C541T	Arg181Cys	Unknown	germline
7	G715A	Glu239Lys	Kinase	germline
8	A751T	Ile251Phe	Kinase	germline
9	G954A	Arg318His	Kinase	germline
10	G961A	Glu321Lys	Kinase	somatic
11	A967C	Thr323Pro	Kinase	germline
12	C1427A	Thr476Lys	Kinase	germline

a) We first introduced above point mutations in the CHK2 coding sequence into the Mammalian expression plasmid encoding HA-tagged CHK2 using the QuickChange Site-Direct Mutagenesis Kit (Invitrogen) for the generation of expression constructs encoding each mutants. The mutant constructs were confirmed by sequence analysis. GST-Cdc25C, containing the C-terminal fragment (residues 200-256) of Cdc25C, was used as a CHK2 substrate.

b-c) To determine CHK2 kinase activity more accurate and to make the assay possible before and after irradiation, we performed kinase assay in mammalian system instead in the insect cell line (Sf9). We, therefore, established stable cell lines expressing HA-tagged wild-type or the mutant CHK2 constructs in HCT15 cells, which are CHK2 deficient. G418-resistant clones were isolated and exogenous CHK2 expression was confirmed by Western blotting using anti-HA or anti-CHK2 antibody. Only stable cell lines expressing mutant CHK2 at the levels similar to that of wild-type CHK2 were uses for CHK2 kinase activity assay. We successfully generated 9 out of 12 stable cell lines suitable for CHK2 kinase analysis.

d) To examine how these mutations affect CHK2 activation, we examined CHK2 kinase activation before and after DNA damage. Briefly, these cells were irradiated. CHK2 was immunoprecipitated from whole cell extract with anti-HA antibody and CHK2 kinase assays were performed using GST-Cdc25C fragment as substrate. Western blots with anti-phospho-CHK2 T68 and anti-CHK2 antibodies were also performed to examine CHK2 protein level and phosphorylation of CHK2 at Thr-68 site following DNA damage. Compared to wild-type CHK2, the Glu321Lys mutant did not have any detectable CHK2 kinase activity (Figure 1). Interestingly, this mutant still underwent the ATM-dependent phosphorylation of Thr-68 site following DNA



damage. In addition, mutants of Arg117Gly, Arg181Cys, Thr476Lys, Glu64Lys, Glu239Lys, Thr323Pro partially reduced CHK2 kinase activity (Figure 1). Except for the Glu64Lys mutant, none of these CHK2 mutants had a reduction of Thr-68 phosphorylation of CHK2 following DNA damage (Figure 1). These observations are quite different from those seen in the Arg145Pro mutant (2). The Arg145Pro mutant abolishes both CHK2 kinase activity and Thr-68 phosphorylation of CHK2 following DNA damage. Thus, it is likely that these mutations of CHK2 identified in prostate cancer affect some steps in CHK2 activation after the initial phosphorylation of CHK2 at Thr-68 site by ATM.

Key Research Accomplishments:

1. We generated mutant expression constructs and established corresponding stable cell lines for 9 CHK2 mutations identified in prostate cancer patients or tumors. Kinase activity analyses demonstrated that these CHK2 mutations either completely abolished or modest reduced CHK2 kinase activity in comparison with wild-type CHK2 indicating that these CHK2 mutations may induce prostate tumorigenesis due to lack or less kinase activity in the response to DNA damage.

2. We have identified two novel somatic CHK2 mutations in 2/84 prostate tumor samples indicating that CHK2 mutations in prostate cancer could be either germline or somatic. In addition, kinase activity assay demonstrated that one somatic mutation (R117G) had modest reduced CHK2 kinase activity while the other one (E321K) totally abolished CHK2 kinase activity.

Reportable outcomes:

CHKE mutations, both germline and somatic, identified in prostate cancer patients or tumors, impair kinase activity which is necessary for proper responding to DNA damage and for prevention of neoplastic transformation.

Conclusions:

Our results provide first evidence that CHK2 mutations identified in prostate cancer patients or tissues (germline or somatic) may contribute to the development of prostate cancer by altering kinase activity of CHK2 which is critical in responding to the DNA damage and in the maintain genome integrity.

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Appendices:

1. The draft of our manuscript entitled "Characterization of *CHEK2* mutations in prostate cancer" by Xianglin Wu, Xiangyang Dong, Wanguo Liu, and Junjie Chen, which was submitted to Cancer Research. (CHK2 has been named as CHEK2 by HUGO)

Characterization of CHK2 Mutations in Prostate Cancer

Xianglin Wu,[1] Xiangyang Dong,[2] Wanguo Liu,[2] and Junjie Chen[1,3]

[1]Department of Oncology, Mayo Clinic and Foundation Rochester, MN 55905

[2]Division of Experimental Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic/Mayo Medical School, Rochester, MN 55905

Word count

Abstract: 159

Introduction, Results and Discussion: 2530

[3]To whom correspondence should be addressed

Phone: (507) 538-1545

Fax: (507) 284-3906

E-mail: chen.junjie@mayo.edu

Running title:

Chk2 mutants in prostate cancer

Key words: Chk2, prostate cancer, mutation, kinase

ABSTRACT.

The checkpoint kinase 2 (Chk2) is a tumor suppressor that participates in the DNA damage-signaling pathway. It is phosphorylated and activated following DNA damage, resulting cell cycle arrest and apoptosis. Previously, we identified germline mutations of Chk2 in patients with prostate cancer. In this study, we have identified additional two somatic mutations of Chk2 in prostate cancer patients and investigated the functions of these mutants *in vivo*. While most of the germline mutations of Chk2 and one somatic mutation (R117G) have modest reduced Chk2 kinase activity in comparison with wild type Chk2, one somatic mutation (E321K) totally abolishes Chk2 kinase activity. Given that several clinical Chk2 mutations residing in the FHA domain of Chk2, we further analyzed the role of Chk2 FHA domain and demonstrated the requirement of an intact FHA domain for fully activation of Chk2. These results provide evidence that Chk2 mutations identified in prostate cancer may contribute to the development of prostate cancer in some patients.

The abbreviations used are: Chk2, checkpoint kinase 2; SQ, SQ-rich; IR, ionizing radiation; FHA forkhead-associated; HA, hemagglutinin; DOD, department of defense; SPORE, specialized program of research excellence.

INTRODUCTION

Maintenance of genomic integrity depends on the coordination of cell cycle checkpoints, repair systems and apoptosis (1). DNA damage checkpoints play important roles in maintaining genomic integrity following stresses. Mutations in genes involved in these checkpoint pathways, such as p53 and ATM, result in genomic instability and cancer predisposition (2).

Checkpoint kinase 2 (Chk2) is a major downstream effector of ATM. As a protein kinase involved in the DNA damage response, Chk2 is rapidly phosphorylated at Thr68 and activated in an ATM-dependent manner following IR (3, 4). This in turn mediates p53 responses (5-7). Chk2 directly phosphorylates p53 *in vitro*, and possibly mediates p53 stabilization following DNA damage (6, 8). Chk2 also phosphorylates breast cancer tumor suppressor 1 (BRCA1). The phosphorylation of BRCA1 by Chk2 is important for the ability of BRCA1 to restore cell survival after DNA damage (9). Recent findings suggest that Chk2 also regulates PML (10) and E2F-1 (11). These phosphorylation events may contribute to a p53-independent apoptosis pathway following DNA damage.

Several studies suggest that Chk2 is a tumor suppressor. Heterozygous germline mutations in Chk2 were identified in a subset of Li-Fraumeni syndrome patients with wild-type p53 (12), suggesting that mutation of either p53 or Chk2 is sufficient for development of Li-Fraumeni syndrome. Similarly, while p53 mutations are rare in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) (13), a recent study identified a Chk2 mutation in FHA domain and another mutation in Chk2 kinase domain in same patient (14). In 41 bone marrow samples from individuals with myelodysplastic syndrome (MDS), Hofmann et al (15) found one patient with a Chk2 mutation at codon 507 of Chk2. A mutation which leads to impaired Chk2 kinase activity was also found in a lung cancer patient. (16). There are additional Chk2 mutations identified from patients with carcinoma of the breast, colon, lung,

bladder, ovary, and vulva (17). Furthermore, we previously reported that Chk2 may also be mutated in a number of prostate cancer patients (18). Taken together, these findings suggest that Chk2 is a tumor suppressor.

Although many Chk2 mutations have been found in patients, only a few of these mutations has been further characterized. To further understand the contribution of Chk2 mutations in prostate cancer development, we examined all of the known Chk2 mutations identified in prostate cancer patients (18). These studies suggest that most of these mutations affect Chk2 kinase activity, supporting a role for Chk2 in the development of prostate cancer.

MATERIALS AND METHODS

Tissues and cell lines for mutation screen Eighty-four primary prostate tumor tissues were analyzed in this study. Tumors in this set were unselected and collected between 1997 and 1998 in Mayo Clinic. Surgical specimens were collected from patients between 48 and 75 years of age. Matched normal adjacent prostate tissues were used to determine whether the mutations identified are somatic or germline. Three commonly used prostate cancer cell lines (LnCaP, PC-3, and DU-145) were also included in this study.

Genomic PCR and Mutation Analyses DNA isolation from tumor tissues and cell lines was performed following the manufacturer's protocol (QIAGEN). Thirteen pairs of intronic primers covering 14 exons of the Chk2 gene (Genbank No. XM_009898) were designed (available upon request). Primers used for amplification of exons 10-14 were particularly designed so that either one or both primers for each set of primers had a base mismatch in the most 3' nucleotide compared with sequences from nonfunctional copies of Chk2. The primers thus preferentially amplified the functional Chk2 on chromosome 22 rather

than nonfunctional elsewhere in the genome. PCR amplification was done in a volume of 12.5 ml containing 25 ng of genomic DNA, each primer at 0.2 mM, each dNTP at 0.2 mM, 2.0 mM MgCl2, 0.5 U of *Taq* polymerase (AmpliTag Gold, Perkin Elmer), and 1 X buffer provided by the manufacturer. Denaturing high-performance liquid chromatography (DHPLC) analyses and direct sequenceing of the PCR products were performed as previously described. (19)

Constructs Mammalian expression plasmid encoding HA-tagged Chk2 was described earlier (20). QuikChange site-directed mutagenesis kit (Strategene) was used to introduce point mutations in the Chk2 coding sequence for the generation of expression constructs encoding Glu64Lys, Arg117Gly, Arg 145Pro, Ile157Thr, Arg181Cys, Glu239Lys, Thr323Pro, Thr321Lys, and Thr383Ala mutants. GST-Cdc25C, containing the Cterminal fragment (residues 200-256) of Cdc25C, was used as a Chk2 substrate (21).

Cell lines and culture conditions All cell lines were obtained from American Tissue Culture Collection and cultivated in RPMI 1640 supplemented with 10% fetal bovine serum. To establish stable cell lines expressing HA-tagged wild-type or mutant Chk2, HCT15 cells were cotranfected with plasmids encoding the indicated HA-tagged wildtype or mutant Chk2 and pcDNA3. G418-resistant clones were isolated and exogenous Chk2 expression was confirmed by Western blotting using anti-HA or anti-Chk2 antibody. Clones with similar expression levels of Chk2 were selected for this study. The stable cell lines were cultivated in RPMI 1640 supplemented with 10% fetal bovine serum plus 100 microgram/ml G418.

Antibodies The generation of anti-Chk2 monoclonal antibody, rabbit-derived anti- Chk2 polyclonal antisera (20) and anti-Chk2pT68 antibody were reported previously (21).

***In vitro* Chk2 kinase assay** HA-tagged wild-type and mutant Chk2 were immunoprecipitated with anti-HA antibodies from extracts of cell lines stably expressing wild-type or various mutants Chk2. For Chk2 kinase assay, immunoprecipitated Chk2 was incubated with substrates for 30 min at 30°C in 30 ml of kinase buffer (50 mM Tris pH 7.5, 10 mM MgCl2, with 10 mM ATP and 10 mCi of 32P-ATP). The reactions were stopped by the addition of 30 ml of 2′ laemmli's SDS sample buffer. Proteins were separated by 12.5% SDS-PAGE and transferred onto PVDF membrane. 32P incorporation in substrates was visualized by autoradiography.

RESULTS

Chk2 mutations were identified in patients with prostate cancer.

To understand Chk2 is involved in the development of prostate cancer, we screened mutations in Chk2 gene in patients with prostate cancer. In 84 unselected primary prostate tumor samples, we identified a total of 9 mutations. Seven were considered to be germline or early somatic mutations since they were also present in their matched normal prostate tissues (18) (table 1). However, the remaining 2 mutations (A349G and G961A) were only present in tumors but not in matched normal prostate tissues, and were considered to be somatic Chk2 mutations (table 1). In addition, these two mutations were not present in 95 normal control DNA samples (data not shown) nor in a total of 1,000 of genomic DNA samples isolated from affected or unaffected men (18). These two somatic mutations changed the conserved amino acids in the Chk2 protein, Arg117Gly (A349G) and Glu321Lys (G961A), raising the possibility that these mutants may affect Chk2 function in the cell.

Chk2 mutants in prostate cancer affect Chk2 kinase activity.

To examine whether these Chk2 mutations identified in prostate cancer affect Chk2 function, we introduced these mutations into Chk2 expression vector using QuikChange site-directed mutagenesis. To facilitate the study of these Chk2 mutants, we generated cell lines stably expressing HA-tagged wild-type and mutant Chk2 in HCT15 cells, which are Chk2 deficient (12). Only cell lines expressing mutant Chk2 at levels similar to that of wild-type Chk2 were used for analyses.

To examine how these mutations affect Chk2 activation, we examined Chk2 kinase activation before and after DNA damage. Briefly, these cells were irradiated and collected one hour later. Chk2 was immunoprecipitated from whole cell extract with anti-HA antibody and Chk2 kinase assays were performed using GST-Cdc25C fragment as substrate. Western blots with anti-phospho-Chk2 T68 and anti-Chk2 antibodies were also performed to examine Chk2 protein level and phosphorylation of Chk2 at Thr-68 site following DNA damage. Compared to wild-type Chk2, the Glu321Lys mutant did not have any detectable Chk2 kinase activity (Figure 1A). Interestingly, this mutant still underwent the ATM-dependent phosphorylation of Thr-68 site following DNA damage. In addition, mutants of Arg117Gly, Arg181Cys, Thr476Lys, Glu64Lys, Glu239Lys, Thr323Pro partially reduced Chk2 kinase activity (Figure 1). Except for the Glu64Lys mutant, none of these Chk2 mutants had a reduction of Thr-68 phosphorylation of Chk2 following DNA damage (Figure 1). These observations are quite different from those seen in the Arg145Pro mutant. The Arg145Pro mutant abolishes both Chk2 kinase activity and Thr-68 phosphorylation of Chk2 following DNA damage (20). Thus, it is likely that these mutations of Chk2 identified in prostate cancer affect some steps in Chk2 activation after the initial phosphorylation of Chk2 at Thr-68 site by ATM.

Generation of Chk2 mutations in the FHA domain of Chk2.

The regulation of Chk2 activation following IR has been well studied. In response to irradiation, Chk2 is first phosphorylated at Thr-68 by ATM (3, 4, 22). The phosphorylated Thr-68 site of Chk2 interacts with the FHA domain of another Chk2 molecule, and thus leads to the formation of Chk2 oligomers (23) (24). It is speculated that Chk2 oligomerization may further regulate Chk2 activation. One way to further activate Chk2 is through autophosphorylation of Chk2. Two of the Chk2 autophosphorylation sites are Thr-383 and Thr-387, located within the activation loop of the Chk2 kinase domain (25). Mutation of these two residues (Thr to Ala) abolishes Chk2 activation (24), indicating that autophosphorylation of Chk2 directly affect Chk2 kinase activity. In addition, we and others have identified Ser-516 of Chk2 as an additional Chk2 autophosphorylation site (26, 27). Mutation of this residue (Ser-516-Ala) reduces Chk2 kinase activity and impairs Chk2-dependent apoptosis following DNA damage (27). Thus, there seems to be an elaborate regulation of Chk2 activation following DNA damage, and the FHA domain of Chk2 seems to be involved in this complex regulation.

Recent studies suggest that the FHA domain is a phospho-protein binding domain (28, 29). Because of this biochemical activity, it is speculated that the FHA domains may play critical roles in signal transduction. The FHA domain of Chk2 is conserved from yeast to mammals. To further study the role of FHA domain in Chk2 activation, we generated several additional missense mutations in Chk2 FHA domain (Table 2; G116A+R117A, S140A+H143A, N171A). Based on the structure of the Chk2 FHA domain (28, 29), all these residues are presented on the conserved phospho-protein binding surface. Residues R117 and S140 are proposed to be directly involved in the interaction between the FHA domain and phospho-peptides (29). Another set of mutations within the Chk2 FHA domain are CEYCFD-to-NAAIRS and GPKNSY-to-NAAIRS (Table 2). They localize at the variable-loop surface of the Chk2 FHA domain (28, 29). The functional significance of these variable loops is not yet clear.

We chose the sequence NAAIRS because it is thought to be a flexible linker based on its appearance in both alpha-helical and beta-sheet structures (30).

Examine the role of FHA domain in the regulation of Chk2 kinase activity

Similar to that described above for the analysis of Chk2 mutations identified in prostate cancer, we generated HCT15 stable cell lines expressing HA-tagged Chk2 containing various mutations in the FHA domain. While wild-type Chk2 was activated following DNA damage, the G116A+R117A mutant completely abolished Chk2 kinase activity, as measured by the mutant protein's ability to autophosphorylate or phosphorylate its substrate Cdc25C (Figure 2A). Surprisingly, none of the other mutants in the FHA domain, S140A+H143A, N171A, CEYCFD-NAAIRS and GPKNSYNAAIRS, affected Chk2 kinase activity (Figure 2). These mutants also did not affect the phosphorylation of Thr-68 site following DNA damage (Figure 2).

DISCUSSION

Chk2 is a tumor suppressor involved in the development of several human cancers. We have previously identified Chk2 mutations in prostate cancer patients. Here, we examined whether these Chk2 mutations affect Chk2 activation in vivo. In this report, we have shown that most of these Chk2 mutations reduce or abolish Chk2 kinase activity, suggesting that these Chk2 mutations may contribute to the prostate cancer development.

Chk2 contains three domains, the SQ/TQ, FHA, and kinase domains. SQ/TQ consensus sites are sites phosphorylated by ATM/ATR (3, 22). One of the critical phosphorylation sites is the Thr-68 site. Phosphorylation of Thr-68 is important for Chk2 activation and oligomerization. So far, four Chk2

mutations have been identified in the SQ/TQ region (17). It has not been determined how these mutations affect Chk2 activity. We identified a Chk2 mutation, Glu64Lys, in the SQ/TQ region in a patient prostate cancer. This mutation reduces both phosphorylation of Chk2 at the Thr-68 site and Chk2 kinase activity following IR (Figure 1B). It is likely that the Glu64Lys mutation might change the conformation of the Chk2 SQ/TQ domain and thus reduce the ability of ATM to phosphorylate and activate Chk2.

We also identified 4 mutations of Chk2 within the kinase domain of Chk2. While the Glu321Lys mutant completely abolishes Chk2 kinase activity following IR, the other three mutants, Glu239Lys, Thr323Pro, Thr476Lys, partially reduce Chk2 kinase activity. This is similar to an early report that the D311V mutant of Chk2 identified in lung cancer only reduces Chk2 kinase activity by 50% (16). These findings suggest that impairment of full activation of Chk2 might contribute to tumorgenesis. The FHA domain of Chk2 is highly conserved from yeast to mammals. Rad53, a yeast homology of Chk2, associates with checkpoint protein Rad9 through its FHA domain following DNA damage (31-33). This FHA domain-mediated interaction is important for Rad53 activation (31). In mammals, Chk2 forms oligomers through interaction of the FHA domain of one Chk2 molecule and the pT68 site of another Chk2 molecule (23, 34). Therefore, it appears that the FHA domain is essential for the tumor suppressor function of Chk2. Arg145Pro and Ile157Thr mutations in the Chk2 FHA domain have been previously identified (12). The Arg145Pro mutation destabilizes the mutant Chk2 protein, reducing its half-life from >120 min to 30 min (35). In addition, the Arg145Pro mutant also abolishes Chk2 kinase activity and Thr-68 phosphorylation following DNA damage (20). In contrast to the Arg145Pro mutant, we did not detect any defects in the Ile157Thr mutant (20). However, other groups have reported that the Ile157Thr mutant has defects in the regulation of BRCA1 (29), CDC25A (36) and p53 (37). In this study, we characterized an additional FHA domain mutation identified in prostate cancer patients,

Arg117Gly. Arg117 is a conserved residue in the FHA domain and is proposed to be required for the phospho-peptide binding activity of the Chk2 FHA domain (29). An study suggested that the Arg117Ala mutation abolishes Chk2 oligomerization (34). Indeed, we have shown that the clinical Arg117Gly mutation has greatly reduced Chk2 kinase activity after IR. In agreement with this observation, the Gly116Ala/Arg117Ala double mutation completely abolishes Chk2 kinase activity, suggesting that these are critical for Chk2 function. Surprisingly, several other mutants in the conserved or variable regions of the FHA domain did not affect Chk2 kinase activity. It is possible that while these mutants do not affect Chk2 kinase activity, they may affect the interaction of Chk2 with other cellular proteins and thus influence the DNA damage responses.

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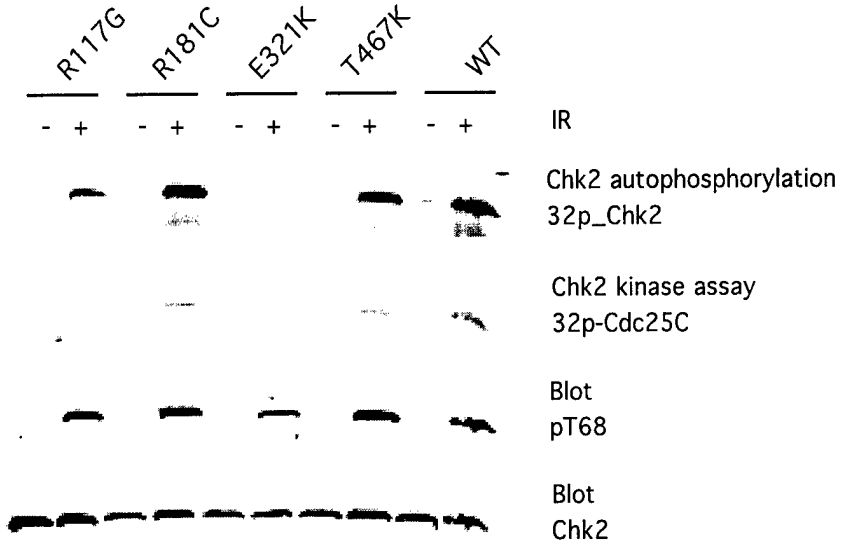
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Blot
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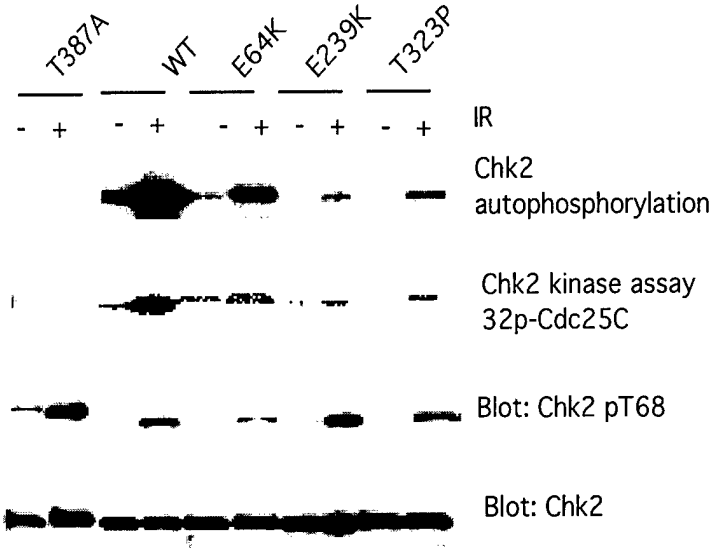
1B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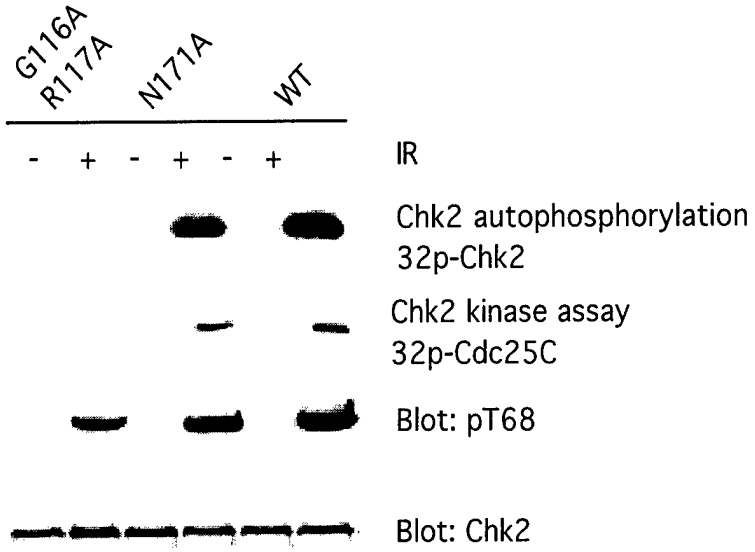
2A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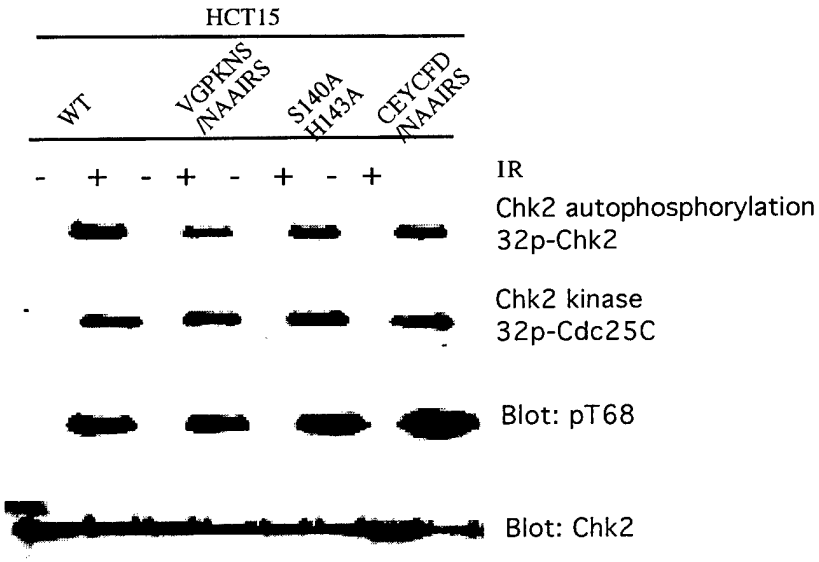
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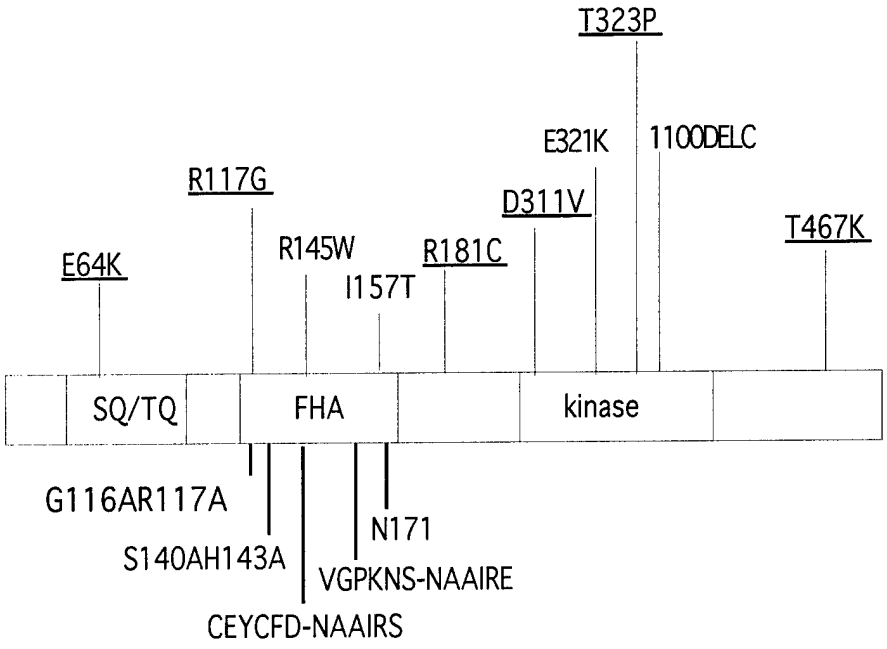
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S140AH143A
VGPKNS-NAAIRE
CEYCFD-NAAIRS

Table 1

Chk2 mutations in prostate cancer and FHA domain of Chk2

mutants	Amino acid change	Domain	
1	Glu64Lys	S/TQ-rich	germline
2	Arg117Gly	FHA	somatic
3	Arg145Pro	FHA	germline
4	Ile157Thr	FHA	germline
5	Arg181Cys	Unknown	germline
6	Glu239Lys	Kinase	germline
7	Glu321Lys	kinase	somatic
8	Thr323Pro	Kinase	germline
9	Thr476Lys	Kinase	germline

Table 2

Chk2 mutations within FHA domain of Chk2

mutants	Amino acid change	Domain
1	N171A	FHA
2	S140AH143A	FHA
3	G116AR117A	FHA
4	CEYCFD-NAAIRS	FHA
5	VGPKNS-NAAIRE	FHA